Annual Report

of the

COMMISSION ON NEUROTROPIC VIRUS DISEASES

of the

Board for the Investigation and Control of

Influenza and Other Epidemic Diseases in the Army

in the Preventive Medicine Service of the Office of The Surgeon General

covering the period of April 1st, 1944, to March 31st, 1945

prepared by

John R. Paul, M.D.
Director

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AREAS OF WORK AND PERSONNEL

The investigative work of the Commission is now being carried out at four stations:

1) Princeton, N. J. The dengue and sandfly fever laboratory, located at the Rockefeller Institute for Medical Research at Princeton, has been operating with the following staff: Lt. Col. A. B. Sabin, M.C., Director; Lt. R. W. Schlesinger, M.C.; Capt. W. G. Jahnes, Sn.C.

2) San Francisco, Cal. "Encephalitis" laboratory at the G. W. Hooper Foundation, University of California, Dr. W. McD. Hammon, Director; Dr. W. C. Reeves and W. N. Mack.

3) New York City. The encephalitis laboratory at the Rockefeller Institute for Medical Research, Dr. P. K. Olitsky, Director; Dr. J. Casals.

4) New Haven, Conn. Yale University School of Medicine:

a) The hepatitis laboratory under Maj. W. P. Havens, Jr., M.C.

b) The poliomyelitis and encephalitis laboratory under Drs. Robert Ward and J. L. Melnick.

During the past year two new members have been added (during 1944-45) to the Commission. These include Captain (now Major) W. P. Havens, Jr., M.C., and Lieutenant R. Schlesinger, M.C.

Lt. (now Capt.) William G. Jahnes, Sn.C., was also assigned to the Commission as a member of Colonel Sabin's staff.

Promotions.- The following Army members and assistants of the Neurotropic Virus Disease Commission have been promoted during the past year:

1) Capt. W. P. Havens, Jr., M.C., to Major.

2) Maj. A. B. Sabin, M.C., to Lieutenant Colonel.

3) Lt. W. G. Jahnes, Sn.C., to Captain.

4) Maj. Murray Sanders, M.C., to Lieutenant Colonel.

ACTIVITIES IN 1944-45

This Commission is primarily organized to study neurotropic virus diseases, but its activities have also included work on other virus diseases of importance to the Army, which require investigation which is not completely covered by the work of other Commissions or Governmental groups. Specifically these virus diseases include:

Sandfly fever

Dengue

Infectious Hepatitis

Our work with infectious hepatitis has been maintained in collaboration with two other Commissions working under the Board, namely the Commission on Measles and Mumps, and the Commission on Influenza. This has proven to be a satisfactory arrangement.

Dengue.- This important military disease has been extensively studied by Colonel Sabin. He has worked with strains of dengue virus received in serum from acute cases in soldiers from (a) an outbreak in Hawaii early in 1944, and (b) from New Guinea in April and May 1944. His work has been made possible by the collaboration of the following agencies:- The Rockefeller Institute for Medical Research of Princeton; The New Jersey State Prison of Trenton, N. J.; The Department of Institutions and Agencies State of New Jersey.

Over 200 volunteers have been used in this work on the experimental disease in man. The following aspects of this disease have received attention:

1) The persistence of virus in blood throughout the febrile period of the disease.

2) The capacity of various species of mosquitoes prevalent in the

U.S.A. to transmit dengue.

3) The effect of prophylactic doses of atabrine on the course of dengue which has been found to be negligible.

4) The effect of yellow fever vaccination on the course of dengue.

5) The interference between dengue and viscerotrophic yellow fever virus in rhesus monkeys and Aedes egypti mosquitoes.

6) The development of neutralizing antibodies in dengue and the study of multiple strains of the virus.

7) Cross-neutralization tests have been made with the New Guinea and Hawaiian strains. These have indicated the existence of distinct immunological types of dengue virus.

8) Of practical importance have been the attempts to produce the immunity by inactivated virus. The use of ultraviolet light and convalescent serum-virus mixtures have yielded negative results.

9) The production of a modified form of the disease by the intranasal instillation of the virus (as compared with the subcutaneous injection) has produced a mild (modified) disease which confers immunity.

10) More important, however, has been the successful propagation of this virus in mice - the first time that this has been accomplished. By adaptation to mice the virus has been modified; the disease produced in man by the mouse-adapted strain is mild, but it also confers immunity on the inoculated individual. These experiments are still in progress.

Japanese B Encephalitis Vaccine.- In 1942-43, Col. Sabin and his colleagues working under the Commission in Cincinnati, developed a mouse brain vaccine against Japanese B encephalitis. A demand for the production of this vaccine on a large scale for Army and Navy use, has recently been made. Part of the supervision for this production has been in the hands of Col. Sabin. In preparation for the production of the vaccine, workers in various biological houses have been vaccinated and considerable further information has been

gained as to the production of neutralizing antibodies against Japanese B encephalitis virus following vaccination.

Dr. Robert Ward and other members of the Commission have prepared an outline on the Epidemiology of Japanese B encephalitis and indications for the possible use of a vaccine in the control of this disease.

Infectious Hepatitis.- At the Hepatitis Laboratory at Yale Major Havens has also studied this disease experimentally in man. More than 50 human volunteers have been inoculated and the experimental disease has been repeatedly reproduced. This work has been made possible by the collaboration of the following agencies: Yale University School of Medicine; Selective Service; the Bureau of Personnel, State of Connecticut; the Middletown and Norwich State Hospitals of Connecticut; the Federal Correctional Institution of Danbury, Conn.; the U. S. Public Health Service; and the National Association for Religious Objectors. For the experiments we have used volunteers who were Conscientious Objectors serving in State institutions, and prisoners. Following the lead of Commission on Measles and Mumps we have also established and maintained a special group of 27 Conscientious Objectors in New Haven, all of whom have volunteered for these experiments.

The most important findings which have come out of these experiments have been the demonstration that the disease can be produced by feeding feces from a case of infectious hepatitis. This confirms a British report of experiments made independently a few weeks prior to our experiments. Similarly the disease can be produced with a short incubation period (about 30 days) by feeding serum. Like the icterogenic agent of the serum or plasma jaundice, the "virus" of infectious hepatitis has been shown to resist heat for half an hour (56° C.) and to be filterable. Several comparative studies on the agent causing homologous serum jaundice and causing infectious hepatitis have been made.

Cross immunity studies have been carried out in which men convalescent from serum jaundice have been re-infected with infectious hepatitis.

No evidence of immunity to infectious hepatitis has been detected in these individuals convalescent from serum jaundice.

Clinical studies of the experimental disease are also in progress.

Attempts to propagate the virus in chick embryos and experimental animals have again proved negative.

Following the demonstration by Dr. Stokes and Capt. Neefe of the Commission on Measles and Mumps, that gamma globulin will protect injected individuals against infectious hepatitis, this substance has been tried in two epidemics:- (a) at a girls' school in Providence, R. I., and (b) at a Catholic Institution for children in New Haven. In the first experiment (a) the epidemic was too brief for a test; in the second (b) evidence of protection of the inoculated children was demonstrated, thus confirming Dr. Stokes' results.

Poliomyelitis.- Although poliomyelitis has not been a prominent disease of the Army, outbreaks - for the most part in overseas troops - have occurred. An experiment carried out by Dr. Robert Ward at the Yale University Laboratory, which has only been in part a Commission project, will be reported at this time. It concerns the natural infection of food within an epidemic area and may well have bearing on the manner in which poliomyelitis is spread.

The experiment was carried out as follows: During the serious outbreak of poliomyelitis in North Carolina during the summer of 1944, food -- mostly in the form of bananas -- was placed on plates in and about the homes of cases of poliomyelitis. Flies had free access to this food and the problem was: Could flies contaminate this food so that when it was eaten, infection might result? The food was frozen on dry ice, transported to New Haven, and fed to two chimpanzees. Both animals which ate this food promptly became intestinal carriers of the virus over a period of several weeks.

Epidemics of poliomyelitis among civilian populations studied during the past year include those of western North Carolina, New York City, western New York State, and north central Pennsylvania.

Further studies on mouse-adapted strains of poliomyelitis described in previous annual reports have been carried out. Dr. Robert Ward has developed antigens from mouse brains which have proven satisfactory for a complement fixation test. This is the first time that complement fixation tests for multiple strains of mouse poliomyelitis have been successfully achieved. It should be mentioned however, that when these strains become adapted to the mouse their immunological capacity changes so that they are sufficiently different from the parent strain that no cross complement fixation reaction occurs. There is little hope therefore that this particular test will be valuable as a diagnostic test in human poliomyelitis as yet.

The use of vervet monkeys imported from East Africa was tried in our laboratory following up the work of last year when these animals were used by our Commission in Egypt. A successful isolation of poliomyelitis virus from flies was accomplished in the vervet monkey. Heretofore this has only been accomplished (with one exception) in the Cynomolgus monkey -- a species of monkey which is peculiarly susceptible to poliomyelitis infection.

Material from a poliomyelitis outbreak in the Philippine Islands among troops has been studied. This material included specimens of serum, central nervous system tissue (from fatal cases), feces and throat washings. Poliomyelitis virus has not as yet been isolated from this material. It is still being investigated.

Western Equine Encephalomyelitis and St. Louis Encephalitis.- Dr. Hammon's work in San Francisco has been carried on with the collaboration of the George Williams Hooper Foundation and the State of California.

During previous years part of these investigations have been carried on under an OSRD contract. As of July 1st, 1945, all his work on the arthropod vectors in encephalitis will be transferred to the Commission.

Each year these epidemiological and ecological studies have brought forth new facts about the spread of these diseases. A total of about 181,000 blood sucking arthropods has been tested for virus infection in these studies;

74 strains of W.E.E., and 7 strains St. L.E. viruses have been isolated from mosquitoes in three western states. The mosquitoes involved have represented 6 species and 4 genera. Experimentally, W.E.E. virus has been transmitted in Dr. Hammon's laboratory by 4 new species (3 genera), and St. L.E. by 10 species (3 genera). The feeding habits of several species of mosquitoes were studied and Culex mosquitoes in particular were correlated with bird reservoirs. The neutralization tests made on 925 animal sera have further proved the importance of birds as vertebrate hosts.

Japanese B encephalitis virus has been transmitted experimentally by 6 species (3 genera) of the California mosquito. The chicken has been demonstrated as a suitable reservoir of Japanese B virus.

Isolations of 3 strains of a new encephalitic virus from California mosquitoes has been made. Dr. Hammon has also studied the possible transmission of W.E.E. and St. L.E. by ticks and has been unable to confirm the work of Dr. Margaret Smith that the chicken mite can transmit St. L.E. virus.

Epidemic areas studied in 1944 intensively include: Kern County, California; the Yakima Valley, Oregon; and Oklahoma.

Poliomyelitis outbreaks investigated include that in Occidental College, Los Angeles, and in the U. S. Navy Receiving Barracks, Portland, Oregon.

Dr. Hammon has also carried out some work on experimental dengue in man using the strains from the southwest Pacific (sent by Col. Philip). He has studied the ability of California species of mosquitoes to transmit the virus to man. Three California species have thus far failed to successfully transmit this virus.

W.E.E. Vaccine. It has been the opinion of the Commission that vaccination against Western equine encephalomyelitis be chosen as a model for the formulation of opinions regarding vaccination against other types of seasonal or arthropod-borne encephalitides. No new information has been obtained during 1944-45 about the efficiency of the vaccines now available, but the Commission has prepared a report bringing up to date its views on the

subject, and recording the amount of available vaccine now present in this country for possible use in the coming summer.

Immunological Studies with Encephalitic Viruses in Experimental Animals.- At the Rockefeller Institute Drs. Olitsky and Casals have vaccinated mice with Russian tick-borne encephalitis virus and have studied the antibody response as well as resistance to infection. These studies reveal that immunity following vaccination with the Russian virus is correlated with neutralizing antibody in the serum, when this antibody is determined by intraperitoneal inoculation of serum-virus mixtures, but not when determined by intracerebral mixtures.

In the same laboratory Dr. Casals has recently developed for the first time a complement fixation test against Venezuela encephalitis virus.

Sera sent for diagnostic purposes from many parts of this country and the globe have been tested by the complement fixation test of Dr. Casals in his laboratory during the past year. As in the past, few of them have yielded positive results but those which have, have been of great significance.

The Technique of Neutralization Tests in Neurotropic Viruses.- In 1942 the Commission proposed a neutralization test for use with certain encephalitis viruses. Much new work and information have transpired in the past two years and the Commission has turned its attention to a revision of this technique described by Dr. Morgan, that complement can enhance the neutralizing capacity of an antiserum against W.E.E. virus. New information has also been brought to light about the lability of the antibody and the effects of diluting serum.

Respectfully submitted,

John R. Paul, M.D.

SCIENTIFIC REPORTS

(Unpublished but prepared specifically for and submitted to the S.G.O.)

April 1st, 1944 - March 31st, 1945

May 1944 — Report of the MIDDLE EAST EXPEDITION of the VIRUS COMMISSION, by Members of the Neurotropic Virus Disease Commission.

June 23, 1944 — Final Report on the Vaccination of Mexican Agricultural Workers, against Western Equine Encephalomyelitis in Kern County, Cal., by Robert Ward, M.D.

An Apparent Food Borne Outbreak of Poliomyelitis among Navy Cadets, by W. McD. Hammon, M.D.

July 1944 — Report of Results of Laboratory Tests on Material Collected in or Received from Nebraska in the Summer of 1943, by W. McD. Hammon, M.D.

Jan. 1945 — Reports on Dengue, by Lt. Col. A. B. Sabin, M.C.

a) Successful propagation of dengue virus in mice.

b) Effect of daily intake of atabrine on clinical course of experimental dengue in human volunteers.

c) Factors responsible for mild, usually rash-free short febrile forms of dengue in New Guinea.

d) Dengue neutralization test on serum of Sgt. Donald G. Aker, ASN 35374353, who had a febrile illness suspected of being dengue.

Jan. 1945 — Isolation of Sandfly-fever-like Virus Immunologically distinct from the Sicilian Sandfly Fever and Hawaiian Dengue Viruses from the Serum of a Patient with a Febrile Illness occurring among personnel of the A.F.HQ. in Italy during the summer of 1944, by Lt. Col. A. B. Sabin, M.C.

Feb. 1945 — Current (1945) Memorandum on Vaccination against Western Equine Encephalomyelitis, by the Commission.

Feb. 1945 — Japanese B Encephalitis. Part I. The Disease. Part II. Control Measures, with particular reference to Vaccination, by the Commission.

Feb. 1945 — Properties of the Agent of Infectious Hepatitis, by Maj. W. P. Havens, Jr., M.C.

Feb. 1945 — Cross-Immunity between Homologous Serum Jaundice and Infectious Hepatitis not Demonstrated, by Maj. W. P. Havens, Jr., M.C.

Dec. 1944 & Feb. 1945 — Preliminary (and Final) Report on the Epidemic of Infectious Hepatitis at the Wheeler School in Providence, R.I., by J. R. Paul, M.D., and C. A. Janeway, M.D.

March 1945 — Preliminary Report on the Prophylactic use of Gamma Globulin in an Epidemic of Hepatitis at the St. Francis Orphan Asylum in New Haven, Conn., by the Commission (J. R. Paul, M.D. and Maj. W. P. Havens, Jr., M.C.).

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Poliomyelitis in British and American Troops in the Middle East, by J. R. Paul, W. P. Havens, Jr., and C. E. van Rooyen, Brit. Med. J., 1: 841 (June 24) 1944.

Phlebotomus (Pappataci or Sandfly) Fever. A Disease of Military Importance. Summary of Existing Knowledge and Preliminary Report of Original Investigations, by A. B. Sabin, C. B. Philip, and J. R. Paul, J.A.M.A., 125: 603 (July 1st) and 693 (July 8th) 1944.

Dimethyl Phthalate as a Repellent in Control of Phlebotomus (Pappataci or Sandfly) Fever, by C. B. Philip, J. R. Paul, and A. B. Sabin, War Medicine, 6: 27 (July) 1944.

Infectious Hepatitis in the Middle East. A Clinical Review of 200 Cases seen in a Military Hospital, by W. P. Havens, Jr., J.A.M.A., 126: 17, (Sept. 2) 1944.

Experimental Production of Hepatitis by Feeding Icterogenic Materials, by W. P. Havens, Jr., R. Ward, V. A. Drill and J. R. Paul, Proc. Soc. Exp. Biol. and Med., 57: 206, 1944.

Human Transmission of Infective Hepatitis by the Oral Route, by W. P. Havens, Jr., J. R. Paul, and C. E. van Rooyen, Lancet, 1: 202 (Feb. 17) 1945.

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5. Casals, J., and Schneider, H. A. Natural Resistance and Susceptibility to Russian Spring-Summer Encephalitis in Mice. Proc. Soc. Exper. Biol. & Med., 54, 201-202 (Nov.) 1943.

6. Hammon, W. McD., and Reeves, W. C. Laboratory Transmission of Western Equine Encephalomyelitis Virus by Mosquitoes of the Genera Culex and Culiseta. J. Exper. Med., 78, 425-434 (Dec.) 1943.

7. Casals, J. Neutralizing and Complement-Fixing Antibody Production and Resistance following Vaccination in Experimental Encephalitis Infections. J. Exper. Med., 78, 447-463 (Dec.) 1943.

8. Casals, J., and Webster, L. T. Relationship of the Virus of Louping Ill in Sheep and the Virus of Russian Spring-Summer Encephalitis in Man. J. Exper. Med., 79, 41-44 (Jan.) 1944.

9. Casals, J. Immunological relationships among Central Nervous System Viruses. J. Exper. Med., 79, 341-360 (April) 1944.